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SIRIPHAN SHUNTAWUTTISETTEE : PREPARATION OF THYROXINE CONJUGATES AS IMMUNOGEN FOR ANTISERUM PRODUCTION OF THYROXINE DIAGNOSTIC KIT. THESIS ADVISOR : ASST. PROF. AMORN PETSOM, Ph.D., THESIS CO-ADVISOR WIYADA CHAROENSIRIWATTANA, 75 pp. ISBN 974-584-365-2

In this study, thyroxine-protein conjugates were prepared by using hapten thyroxine reacted with various kind of protein molecules such as : bovine serum albumin, human serum albumin and poly-D-lysine. Synthesis of thyroxine-bovine serum albumin yielded the highest molar ratio of thyroxine per bovine serum albumin i.e. 22:1 at 25^{o}c and 18:1 at 4^{o}c by using thyroxine 10.00 mg(1.29×10^{-5} mole),carbodiimide 4.00 mg(2.09×10^{-5} mole) and bovine serum albumin 0.80 mg (0.12×10^{-7} mole) at pH 9-10. In case of thyroxine-human serum albumin at the same pH by using thyroxine 20.00 mg (2.57×10^{-5} mole),carbodiimide 5.00 mg (2.61×10^{-5} mole) and human serum albumin 0.50 mg (0.07×10^{-7} mole) gave molar ratio of thyroxine per human serum albumin 20:1 at 4^{o}c and 16:1 at 25^{o}c.

To raise antisera from the animals (rabbits) it was found that the dosages of immunogens used for primary injection and for boosters should be differed i.e. 1.0 and 0.2 mg respectively in order to prevent the tempolarily of non-produced antibody condition. This may be mediated by both a T- and a B-cell effect. The time intervals of immunization were important too. It was found that the rabbits immunized every month could produce the antisera whereas the 2 week boost up interval could not yield the satisfactory antisera. Comparisons of responses to each immunogen showed that the group immunized with highest molar ratio of thyroxine-bovine serum albumin (> 20:1) gave the highest titer at 1:400 whereas the group immunized with molar ratio of thyroxine per bovine serum albumin > 10 < 20:1 yielded the highest titer at 1:130. For thyroxine-human serum albumin, the group immunized with the molar ratio of > 10 < 20:1 gave the highest titer at 1:150. In case of the thyroxine-poly-D-lysine, there was no response from the animals although the molar ratio of thyroxine per protein was the highest(200:1). Characterization of the affinity constants for these antisera showed the values of about 10^{8} to 10^{9} M-1.